The Effect of Cytotoxic Particles on Cell Damage Molecule Release and Pattern Recognition Receptor Activation

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Introduction: Nanoparticles (NP) have been incorporated into dental composite matrices, but their role in the biocompatibility of composites is unknown, mainly when composite particles released during polishing procedures may contain unpolymerized monomers known to be cytotoxic [1]. Cytotoxic substances allow the release of cell damage associated molecular pattern recognition molecules (DAMPs), specifically the high mobility group box 1 (HMGB1) protein. Extracellular HMGB1 is known to act as a cytokine-like protein and activate pattern recognition receptors (PRR) in target cells [2]. The objective of this study was to determine if cell supernatants from cytotoxic concentrations of particles contain HMGB1 and activate PRRs in macrophage cells. **Methods:** Using dental finishing techniques, particles were obtained from the nanocomposite Filtek Supreme Plus (nCP) and the microhybrid composite Z250 (mCP) (3M-ESPE, Minnesota, US). Silica Aerosil OX50 (Degussa® Corp., Germany) with mean particle size of 40 nm was used as a model nanoparticle. RAW264.7 macrophage cells were cultured for 48h with various concentrations of particles dispersed in 1% lung surfactant (Survanta) in OPTI-MEM reduced serum media (Invitrogen, Carlsbad, CA). Supernatants were then collected to a) measure lactate dehydrogenase (LDH OD_{450nm}, BioVision), b) detect HMGB1 release (Western blot) and c) activate PRRs in RAW Blue cells, mouse macrophage reporter cells expressing most TLRs (InvivoGen). PRR activation was validated using pig HMGB1 (SHINO TEST, Japan) by measuring the release of a secreted embryonic alkaline phosphatase protein (SEAP OD_{650nm}) in 24h cultures. **Results:** Using SEM analysis, less than 1% of particles in nCPs and mCPs were below 100nm while most particles collected were ~1000nm. Toxicity data analysis indicated a significant effect ($p<.05$) of particle concentration on LDH and HMGB1 release for all three materials. Toxicity rankings based on LDH (Fig. 1) and HMGB1 release (Fig. 2, top) were similar: SiO_2>nCP>mCP. Using 3µg protein (as for HMGB1 detection), supernatants from nCP-300µg/ml and all doses of SiO_2 induced significant expression ($p<.05$) of SEAP (Fig. 2, bottom) relative to the control (0 µg/ml or 1% surfactant). The overall toxicity ranking based on SEAP release was SiO_2>nCP=mCP (Fig. 2, bottom).

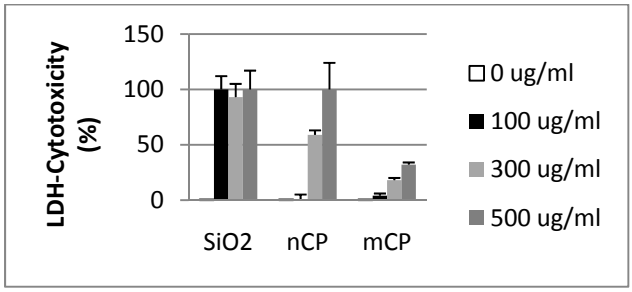

Figure 1. LDH cytotoxicity by particles

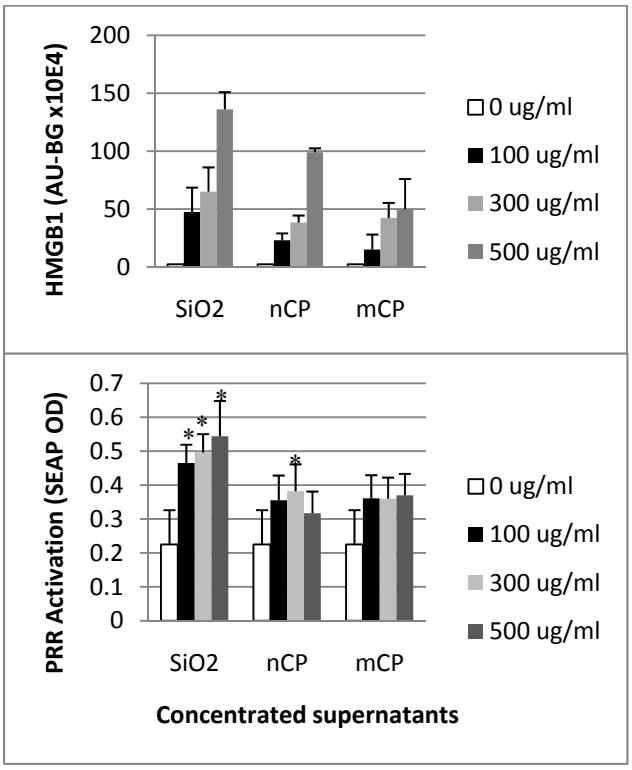

Figure 2. HMGB1-Containing supernatants (top figure) and PRR Activation in macrophage cells (bottom figure)

HMGB1 protein activated PRRs in macrophage cells (SEAP release) in a dose-dependent manner (Table 1).

Table 1. PRR activation (SEAP OD) in cells by pig HMGB1.

HMGB1 (ng/ml)	0	2	4	8	32
SEAP OD Mean	0.093	0.468	0.705	1.497	1.740
(SD)	0.004	0.017	0.112	0.011	0.089

Conclusions: Even though nCPs were more cytotoxic and more HMGB1 releasers than mCPs, most supernatants did not stimulate PRRs. It seems HMGB1 levels once in cell cultures with RAW blue cells were not enough for PRR activation. Further studies are needed to clearly establish the degree of cytotoxicity, the extent of HMGB1 release and PRR activation by composite dust particles. On the other hand, severely cytotoxic concentrations of SiO_2 nanoparticles released HMGB1 in supernatants and activated PRRs in macrophages. The PRR's likely activated by HMGB1 were TLR1/2&4 in the cells [3]. The significance of HMGB1 mediated-PRR activation by cytotoxic particles may be at stimulating innate immune responses for tissue repair or chronic inflammation [3].

References:
1. Hanks CT et al., J Dent Res 2003;83:367-371
2. Klune JR et al., Mol Med 2008;14:476-484
3. Van Beijnum JR et al., Angiogenesis. 2008;11:91-9
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